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Inhibition of phospholipid and platelet-dependent prothrombinase activity in the plasma of patients with lupus anticoagulants

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Summary. Prothrombinase activity was analysed in the plasma of a series of patients with lupus anticoagulants (LAC). In the presence of purified PS-PC (20–80%) vesicles the prothrombinase activity triggered by kaolin was retarded by 2–3 min with respect with normal plasma. The maximal values of prothrombinase activity increased by increasing the amount of phospholipid vesicles. However, in the plasma of the patients they were always lower than those of normal plasma at each phospholipid concentration. Platelet-dependent prothrombinase activity was subsequently investigated. Again, both a delay in appearance and reduced peak values of

prothrombinase activity were observed in the plasma of the patients. This inhibition was partially overcome by the addition of an excess of purified phospholipids. Finally, the effect of LAC IgG on platelet rich plasma-dependent prothrombinase activity was investigated. The main effect observed was a delay of the peak time of prothrombinase activity, while the maximal peaks were affected only by one IgG preparation. We conclude that LAC antibodies can react with both purified negatively-charged phospholipids and platelet procoagulant phospholipids and inhibit prothrombinase activity in a similar way in both cases.

Lupus anticoagulants (LAC) are acquired inhibitors of coagulation described for the first time in 1952 by Conley & Hartmann in two patients suffering from systemic lupus erythematosus (SLE).

LAC are immunoglobulins of the IgG and/or (less frequently) IgM class, which prolong the clotting tests measuring the activated partial thromboplastin time (aPTT) (Mueh *et al*, 1980).

The immunological nature and the mechanism of action of LAC have been extensively studied by the group of Shapiro (Thiagarajan *et al*, 1980; Pengo *et al*, 1987). The purified immunoglobulins react against negatively-charged phospholipids (i.e. phosphatidylserine, phosphatidic acid, phosphatidylinositol and cardiolipin) and they are able to inhibit the binding of factor X and prothrombin to the phospholipid surface, thereby accounting for the prolongation of phospholipid-dependent clotting tests.

Phospholipids are structural components of the platelet membrane. The negatively-charged phospholipids are almost exclusively located in the inner part of the membrane of

resting platelets, but they are exposed on the outer surface upon platelet activation (Bever *et al*, 1982). This transbilayer rearrangement has been called 'flip-flop' and is responsible for the procoagulant properties of platelets in factor X and prothrombin activation (Rosing *et al*, 1985). It seems to be likely that LAC antibodies, by binding to the negatively-charged phospholipids, can interfere with the procoagulant activity of activated platelets. Actually, this has been demonstrated only in one case by Dahlback *et al* (1983), while in their original work Thiagarajan *et al* (1980) were not able to show any inhibitory effect of the LAC immunoglobulin on the binding of factor Xa to platelets. Furthermore, other authors developed assays for the diagnosis of LAC, based on the ability of platelets to normalize the prolonged clotting tests (Firkin *et al*, 1978; Triplett *et al*, 1983). Thus, it was suggested that platelets can by-pass the presence of the acquired anticoagulants. In contrast, it has been suggested that LAC antibodies can react with platelets, because thrombocytopenia (Harris *et al*, 1985), 'ex vivo' platelet activation (Galli *et al*, 1988) and 'in vitro' platelet aggregation abnormalities (Cohen *et al*, 1986) have been reported in patients with the antiphospholipid antibodies. Moreover, it has been observed that commercially available phospholipid reagents for clotting tests, used to detect LAC inhibitors, show a large variation of sensitivity to

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the antibodies due to the variations in both the concentrations and the nature of the phospholipid in the preparation (Mannucci *et al*, 1979). These data led to the hypothesis that LAC antibodies are heterogenous.

To assess whether there is a general reactivity of LAC immunoglobulins towards negatively-charged phospholipids from different sources, we studied the plasma of a series of patients with these antibodies. In particular, we evaluated the inhibitory effect of the acquired anticoagulants on the prothrombinase activity induced by platelets and by purified negatively-charged phospholipids vesicles. We used a computer assisted method, recently developed in this laboratory (Hemker *et al*, 1986), which allowed us to measure the prothrombinase activity in plasma independently of thrombin breakdown by antiproteases. Thus, we could approach closely the 'in vivo' situation. We demonstrate here that the inhibition of prothrombinase activity by LAC antibodies is more dependent on the amount of negatively-charged phospholipids present in the system than on the source of procoagulant surface.

MATERIALS AND METHODS

Patients. Nine patients with LAC were investigated, two males and seven females, 27–60 years old. Details of the clinical history are given in Table I. SLE was diagnosed according to the criteria established by the American Rheumatism Association (Tan *et al*, 1982). Both venous and arterial thrombosis were diagnosed by angiography.

A control group was established of 10 apparently healthy subjects.

Diagnosis of LAC. Blood was collected in trisodium citrate (9 volumes of blood to 1 volume of 0.13 M trisodium citrate) and centrifuged at 4000 *g* for 20 min to obtain platelet poor plasma (PPP). PPP was stored at -80°C until use. Plasma of 10 normal individuals was pooled and used as a reference in the coagulation assays. The following tests were used for the diagnosis of LAC: activated partial thromboplastin time (aPTT) (Activated Thromboplastin, Ortho Diagnostic, Raritan, N.J., U.S.A.), kaolin clotting time (KCT) (Exner *et al*, 1978) and diluted Russell viper venom time (RVV) (Thiagarajan *et al*, 1986) (Wellcome Reagents Beckenham, England), before and after 1:1 mixture of patients plasma with normal pooled plasma, tissue thromboplastin inhibition test (TTI), using Simplastin (General Diagnostics, Warner Lambert Co., Morris Plains, N.J., U.S.A.) diluted 1:50 and 1:500 with saline (Schleider *et al*, 1976).

Preparation of IgG immunoglobulins. IgG immunoglobulins from seven patients and four normal subjects were isolated. Serum (20 ml) was dialysed against 0.02 M K_2HPO_4 , pH 8.0 at 4°C overnight. Then it was applied to a column of DEAE Affi-Gel Blue (Bio Rad, Richmond, Calif, U.S.A.) equilibrated with the same buffer. The effluent immunoglobulins were concentrated by Amicon ultrafiltration, using a PM 10 membrane (Amicon Corp, Lexington, Mass., U.S.A.). Protein concentration was established by a nephelometric method using a Beckman system (Auto ICS, Beckman Instr., Fullerton, Calif., U.S.A.). Immunoelectrophoresis was performed with human antisera specific for total immunoglobulins, IgG,

IgM and light chains (Helena Laboratories, Beaumont, Texas, U.S.A.).

Patients IgG were studied for their anticoagulant activity by adding 0.05 ml of IgG to 0.05 ml of normal pooled PPP in a KCT assay. Anticoagulant activity was considered to be present, when the clotting time was at least 1.5 times that of the control, i.e. with IgG from normal subjects.

Measurement of prothrombinase activity in plasma. For the measurement prothrombinase activity in plasma the procedure of Hemker *et al* (1986) was used. Briefly, 0.24 ml of normal pooled or patients PPP were incubated at 37°C for 5 min with 0.06 ml of Tris buffer (0.1 M NaCl, 0.05 M Tris, pH 7.35) containing kaolin (0.28 mg/ml) and various concentrations of phospholipid vesicles (80% phosphatidylcholine, PC, and 20% of phosphatidylserine, PS), prepared according to Rosing *et al* (1983) or with 0.06 ml of different concentrations of washed platelet suspensions, prepared according to Rosing *et al* (1985). In some experiments, 40 μM of purified PS/PC vesicles were added to PPP at the same time as the washed platelets. Thrombin generation was started by the addition of 0.06 ml of 0.1 M CaCl_2 , pH 7.35. At fixed time intervals 0.01 ml aliquots of the incubation mixture were subsampled into tubes containing 0.465 ml of a Tris-EDTA buffer (0.1 M NaCl, 0.05 M Tris, 0.02 M EDTA, 0.05% ovalbumin, pH 7.9) and 0.025 ml of a 0.004 M solution of chromogenic substrate S2238 (Kabi Vitrum, Stockholm, Sweden) at 37°C . The subsampling tubes were incubated for 2 min at 37°C and then, by addition of 0.3 ml of 50% acetic acid, the reaction was stopped. The amidolytic activity was measured at 405 nm wavelength in a spectrophotometer (LKB-Ultrospec., Bromma, Sweden). From the change in absorbance and the time interval between subsampling and stopping the thrombin amidolytic activity was calculated. Thrombin concentration was calculated using a calibration curve made with active-site titrated human thrombin.

Thrombin breakdown constants were also determined according to Hemker *et al* (1986). Briefly, the same mixture as for thrombin generation curves was used, but with a volume of 0.048 ml of Tris-buffer mixture or platelet suspension instead of 0.06 ml. Two minutes after the thrombin peak 0.012 ml of Soybean trypsin inhibitor (SBTI) (10 mg/ml) (Sigma, St Louis, Mass., U.S.A.) were added to the reaction mixture, so as to stop prothrombinase activity. At variable intervals after the addition of SBTI, 0.01 ml aliquots were subsampled into cuvettes by the use of a time recording pipette. The cuvettes were further treated as described for the thrombin generation test. The overall decay constant is the sum of the α_2 macroglobulin ($\alpha_2\text{M}$) dependent constant (k_2) and the non- $\alpha_2\text{M}$ -dependent constant (k_1), mainly due to antithrombin III. From the ratio of the amount of prothrombin consumed during the experiment (i.e. the total amount of thrombin formed) and the amount taken up by $\alpha_2\text{M}$, determined using staphylocoagulase (50 nm) (Hendrix *et al*, 1983), k_1 and k_2 were determined separately (for details see Hemker *et al*, 1986).

Prothrombinase activity (expressed in nM IIa/min) was calculated by a computer using the thrombin generation data and the experimentally determined decay constants k_1 and k_2 of endogenous thrombin (Hemker *et al*, 1986).

Table I. Clinical and laboratory data in nine patients with LAC

Patient	Age	Sex	Clinical remarks	APTT	APTT 1:1	KCT	KCT 1:1	TTI		RVV	RVV 1:1	Type of LAC antibody
								1/50	1/500			
R.A.	30	F	SLE; transient ischaemic attacks	46	35	186	168	60	119	52	46	—
G.S.	33	M	Peripheral arterial thrombosis	36	36	98	96	50	98	60	49	IgG
C.L.	27	F	Recurrent abortions; deep venous thrombosis	46	37	178	126	54	109	73	46	IgG
D.S.	31	F	SLE	40	—	196	—	61	151	—	—	IgG
M.S.	60	M	Deep venous thrombosis	48	36	130	113	102	252	65	48	—
Z.R.	41	F	Cerebral thrombosis	35	34	109	111	43	85	63	—	IgG
T.A.	29	F	Repeated abortions; SLE	75	66	110	97	59	132	41	38	IgG
T.N.	36	F	Repeated abortions; SLE	34	30	127	100	—	—	—	—	IgG
B.A.	56	F	—	60	43	140	121	—	—	—	—	IgG
Controls (10)				26–30		64–75		35–40	63–77	25–30		

All results are expressed in seconds.

Effect of LAC IgG on prothrombinase activity in normal platelet rich plasma. Platelet rich plasma (PRP) was obtained by centrifugation at 170 *g* for 10 min of freshly drawn citrated normal blood. Platelet concentration was determined with a thrombocounter (Coulter Counter Electronics, Hialeah, Fla., U.S.A.) and adjusted at 300×10^9 platelets/l with autologous PPP.

Thrombin generation and prothrombinase activity were determined according to the method of Béguin *et al* (1989). The samples consisted of 0.24 ml of PRP and 0.06 ml of normal pooled or patients IgG at various concentrations. After 5 min of incubation at 37°C the thrombin generation was started by the addition of 0.06 ml of 0.1 M CaCl₂ or 0.06 ml of a 1:600 dilution in 0.1 M CaCl₂ of human brain thromboplastin, prepared by a modification of the method of Owren & Aas (1951) and prewarmed for 1 h at 37°C. At fixed time intervals 0.01 ml aliquots of the incubation mixture were subsampled into tubes containing 0.465 ml of Tris-EDTA buffer and 0.025 ml of a 0.004 M solution of S 2238 at 37°C. The subsampling tubes were incubated for 2 min at 37°C and then, by addition of 0.3 ml of 50% acetic acid, the reaction was stopped. The amidolytic activity was tested as above described.

In order to overcome the effect of platelet ageing, the experiments were carried out within 2 h from the venipuncture. Prothrombinase activity was calculated as above described.

RESULTS

Coagulation studies and characterization of LAC inhibitors

The aPTT, KCT and dilute RVV clotting times in all the nine patients exceeded those of the normal group and no correction was noted on mixing 1:1 patients and normal PPP. TTI was also abnormal in all the cases (Table I).

Immunoglobulins with LAC activity were found to be IgG in the seven patients in whom the identification of the inhibitor was performed (Table I).

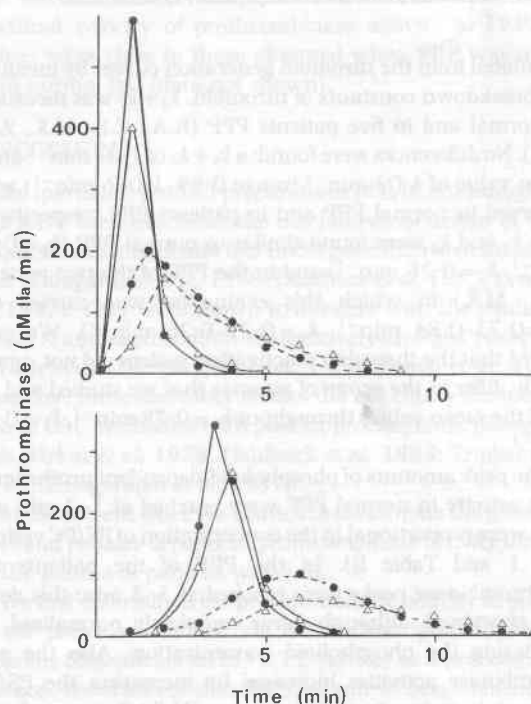


Fig 1. Prothrombinase activity (nM IIa/min) in normal pooled plasma (upper panel) and in the plasma of patient R.A. (lower panel) induced by the following concentrations of PS/PC vesicles: Δ — Δ , 0.5 μ M; \bullet — \bullet , 1 μ M; Δ — Δ , 5 μ M; \bullet — \bullet , 10 μ M.

Effect of phospholipid vesicles and normal platelets on prothrombinase activity in PPP

Phospholipid-dependent prothrombinase activity was evaluated in normal and in eight patients' PPP. Reactions were studied in the intrinsic pathway, using procoagulant phospholipid vesicles (PS/PC) at the concentrations given in Fig 1 and Table II. The course of prothrombinase activity was

Table II. Maximal amounts of prothrombinase activities in patients and normal pooled PPP in the presence of phospholipid vesicles or washed normal platelets

Patient	PS/PC (μM)				Washed platelets ($\times 10^9/\text{l}$)	
	0.5	1	5	10	100	200
R.A.	65	95	251	310	44	53
G.S.	ND*	113	152	ND	37	64
C.L.	76	115	348	415	53	62
D.S.	104	117	316	352	55	75
M.S.	109	204	463	ND	61	104
Z.R.	65	97	305	397	52	85
T.A.	73	123	306	384	65	80
T.N.	ND	ND	ND	ND	ND	ND
B.A.	ND	28	54	73	9	20
Normal pooled PPP	119	200	433	538	81	91

* ND: not done. The data are expressed in nM IIa/min .

calculated from the thrombin generation curves by means of the breakdown constants of thrombin. $k_1 + k_2$ was measured in normal and in five patients PPP (R.A., C.L., M.S., Z.R., T.A.). No differences were found: a $k_1 + k_2$ of 1.04 min^{-1} and a mean value of 1.03 min^{-1} (range $0.99\text{--}1.066 \text{ min}^{-1}$) were observed in normal PPP and in patients PPP, respectively. Also k_1 and k_2 were found similar in normal PPP ($k_1 = 0.78 \text{ min}^{-1}$, $k_2 = 0.26 \text{ min}^{-1}$) and in the PPP of the two patients (C.L., M.S.) in which this evaluation was carried out ($k_1 = 0.73\text{--}0.84 \text{ min}^{-1}$, $k_2 = 0.22\text{--}0.26 \text{ min}^{-1}$). We concluded that the thrombin inactivation system did not significantly differ in the group of plasmas that we studied and we used the mean values throughout $k_1 = 0.78 \text{ min}^{-1}$, $k_2 = 0.25 \text{ min}^{-1}$.

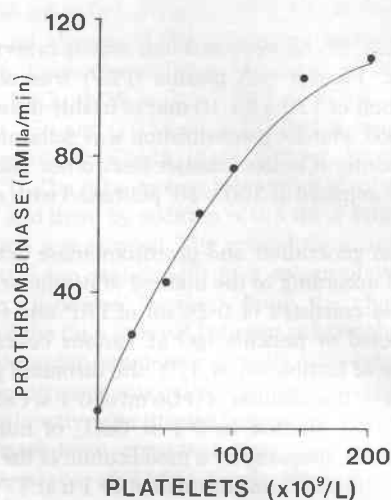
The peak amounts of phospholipid-dependent prothrombinase activity in normal PPP were reached at 1–2 min and they were proportional to the concentration of PS/PC vesicles (Fig 1 and Table II). In the PPP of the patients the prothrombinase peaks were reached at 5–3 min: this delay was shortened, although never completely normalized, by increasing the phospholipid concentration. Also the prothrombinase activities increased by increasing the PS/PC amount; however, in all but one case (M.S.) they were found lower than that of the normal PPP at each phospholipid value (Fig 1 and Table II).

In order to assess differences in the reactivity of LAC inhibitors towards phospholipids of different sources, the prothrombinase activity was subsequently evaluated in the presence of normal platelets as the source of procoagulant phospholipids. The prothrombinase activity was calculated from the thrombin generation data by means of the breakdown constants of thrombin. As a control, k_1 and k_2 were measured in normal PPP, PRP and reconstituted PRP (washed platelets resuspended in autologous PPP at a final number of $200 \times 10^9/\text{l}$). As depicted in Table III, the values of

Table III. Thrombin breakdown constants of normal platelet poor plasma (PPP), platelet rich plasma (PRP) and reconstituted PRP. k_2 is the α_2 macroglobulin dependent constant, k_1 the non α_2 macroglobulin dependent constant.

	k_2 (min^{-1})	k_1 (min^{-1})
PPP	0.13	0.78
PRP	0.13	0.85
Reconstituted PRP*	0.13	0.88

* Washed platelets resuspended in autologous PPP at a final concentration of $200 \times 10^9/\text{l}$.

**Fig 2.** Concentration effect of washed normal platelets on the prothrombinase activity (nM IIa/min) in normal pooled plasma.

thrombin breakdown constants were not influenced by the presence of platelets. Thus, the values of k_1 and k_2 measured in PPP were also used to calculate platelet-dependent prothrombinase activity. A calibration curve was obtained by incubating normal PPP with increasing concentrations of washed platelets (from 0 to $200 \times 10^9/\text{l}$). As depicted in Fig 2, prothrombinase activity increased with the platelet number.

Platelet-dependent prothrombinase activity in normal and eight patients' PPP was subsequently evaluated in the presence of 100 and 200×10^9 platelets/l. Clotting reactions were started by the addition of CaCl_2 . In these conditions the maximal prothrombinase activity was reached after 8–11 min with both 100 and 200×10^9 platelets/l in normal PPP (Fig 3). Also the peak amount of prothrombinase activity did not change significantly with the number of platelets (Table II).

In the plasma of the patients the maximal amounts of

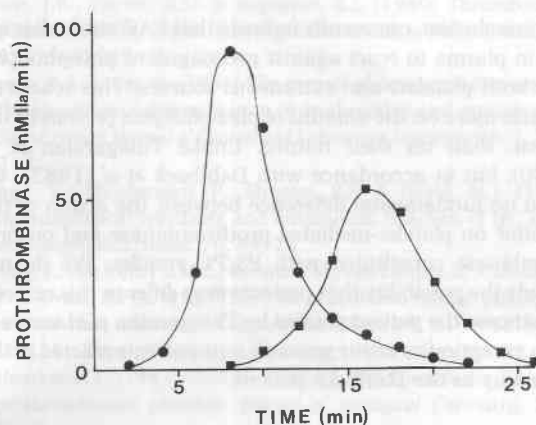


Fig 3. Prothrombinase activity (nM IIa/min) induced by 200×10^9 washed normal platelet/l in normal pooled plasma (●) and in the plasma of patient R.A. (■).

prothrombinase activity were reached later and they were found lower than in normal plasma. The differences were more pronounced in the presence of 100×10^9 platelets/l. In particular, the peak amounts of prothrombinase activity were observed at 10–20 min with 100×10^9 platelets/l and at 9–16 min in the presence of 200×10^9 platelets/l. The values of prothrombinase are given in Table II. When an excess of PS/PC vesicles ($40 \mu\text{M}$) was added at the same time as 100×10^9 washed platelets/l to the plasmas of three patients (R.A., G.S., B.A.) prothrombinase activity was completely normalized in two cases (R.A., G.S.) and partially normalized in the third one (patient B.A.) (data not shown).

Effect of LAC IgG on prothrombinase activity in normal PRP

The maximal prothrombinase activity was reached at 8–11 min when normal PRP was mixed with 10 mg/ml of normal pooled IgG and coagulation was initiated by the addition of CaCl_2 . The peak value of prothrombinase activity was advanced to 6–8 min in the presence of a high dilution of thromboplastin (1:3600 final). No significant differences were found in the maximal prothrombinase activity in these two experiments. In fact, the mean values were $114 \text{ nM IIa min}^{-1}$ (range 94–152) and $95 \text{ nM IIa min}^{-1}$ (range 80–124) in the presence of CaCl_2 and calcium plus thromboplastin, respectively. The main effect exerted by LAC IgG on normal PRP was a delay of the peak time of prothrombinase activity. When the clotting reaction was started by CaCl_2 the peak was reached at 11–18 min. In the presence of thromboplastin the delay was reduced in all cases (range 4–15 min) and completely normalized in two of them (patients C.L. and Z.R.). These results were obtained using 10 mg/ml of IgG with LAC activity. In one case only (patient B.A.) concentrations of IgG higher than 1 mg/ml completely abolished the prothrombinase activity. From Fig 4 it appears that the peak amount was reduced and the lag time was increased by increasing amounts of this patient's IgG. In the other cases the delay of the peak time of prothrombinase activity was almost completely normalized when the concentration of LAC IgG was decreased to 5 mg/ml (data not shown). LAC immunoglobu-

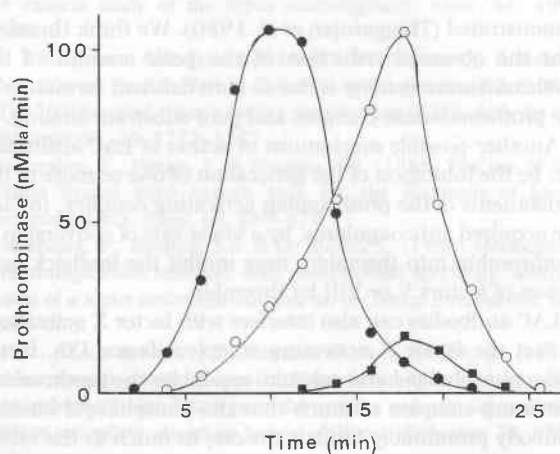


Fig 4. Prothrombinase activity (nM IIa/min) in platelet-rich plasma in the presence of normal and LAC IgG. ●, Normal IgG (10 mg/ml); ■, IgG of patient B.A. (0.5 mg/ml); ○, IgG of patient B.A. (0.1 mg/ml).

lins were, on the contrary, not able to interfere with the maximal velocity of prothrombinase activity in PRP: the values were close to those observed when PRP was mixed with normal IgG (data not shown).

DISCUSSION

Since (partially) purified preparations of LAC immunoglobulins have been available, the mechanism of action of these acquired anticoagulants has undergone intensive investigation (Thiagarajan *et al*, 1980; Dahlback *et al*, 1983; Pengo *et al*, 1987). They were shown to interfere with the binding of factor X and prothrombin to negatively-charged phospholipid surfaces (Thiagarajan *et al*, 1980; Pengo *et al*, 1987). However, these and other studies did not clarify the interaction of LAC antibodies with platelet procoagulant phospholipids (Firkin *et al*, 1978; Dahlback *et al*, 1983; Triplett *et al*, 1983; Thiagarajan *et al*, 1987).

In the present work we wanted to investigate the phospholipid and platelet-dependent prothrombinase activity directly in the plasma of patients with LAC.

We first determined the prothrombinase activity in plasma of the patients activated via the intrinsic pathway, using different concentrations of PS/PC vesicles as a procoagulant surface. We observed that both the rate of development and the peak amount of prothrombinase activity were reduced in the plasma of the patients. This phenomenon was clearly dependent on the amount of phospholipid in the system, since a partial normalization of the prothrombinase activity was reached by increasing the concentration of PS/PC vesicles.

By binding to the phospholipid surface, LAC antibodies may inhibit the prothrombinase activity by hampering the assembly of the prothrombin activating complex (factor Xa, factor Va, phospholipid and calcium) on the procoagulant surface or by hindering the binding of prothrombin to an otherwise normal prothrombinase complex. The interference of LAC immunoglobulins with the binding of factor Xa and prothrombin to purified phospholipids has already been

demonstrated (Thiagarajan *et al*, 1980). We think therefore that the observed reduction of the peak amount of the prothrombinase activity is due to both deficient formation of the prothrombinase complex and poor substrate binding.

Another possible mechanism of action of LAC antibodies can be the inhibition of the generation of one or more of the constituents of the prothrombin activating complex. In fact, the acquired anticoagulants, by a lower rate of conversion of prothrombin into thrombin, may inhibit the feedback activation of factors V or VIII by thrombin.

LAC antibodies can also interfere with factor X activation. In fact the factor X activating complex (factor IXa, factor VIIIa, phospholipid and calcium) resembles the prothrombin activating complex so much that the phospholipid binding antibody presumably inhibits the one as much as the other. This is probably the cause for the observed delay of appearance of the prothrombinase activity. Further studies will have to elucidate the extent of this inhibition.

The influence of LAC on platelet-dependent prothrombinase activity was also investigated. We demonstrated that platelets do not significantly influence the breakdown constants (k_1 and k_2) of thrombin in plasma (see Table III). It has been observed that activated platelets increase the rate of thrombin inhibition by AT III (Jesty, 1985). We did not observe a significant effect. It is noteworthy that no platelet product responsible for the increase of thrombin inhibition has as yet been identified.

We found that prothrombinase activity was also affected in plasma of the patients when washed normal platelets were used as procoagulant surface. Recently, Béguin *et al* (1989) demonstrated the existence of a feedback activation of platelets by the small amounts of thrombin formed in PRP in the early stages of coagulation triggered by tiny amounts of thromboplastin; this makes platelet procoagulant phospholipids available to stimulate a burst of thrombin formation. LAC antibodies, by binding to the exposed negatively-charged platelet phospholipids, hamper this feedback activation, thus accounting for both the delay in the appearance and the reduction of the peak levels of the prothrombinase activity. As expected from the observed correlation between the number of platelets and prothrombinase activity (see Fig 2), this inhibition of platelet-dependent prothrombinase activity was more obvious at a low platelet number. Moreover, it was (partially) overcome by the addition of an excess of phospholipids, thus suggesting that the amount of negatively-charged phospholipid more than its source imports for the expression of the anticoagulant effect of LAC antibodies. The correlation observed in PRP between the delay of appearance of prothrombinase activity and the amount of LAC IgG in the presence of different triggers of platelet activation supports this view. Unlike the patients' plasma, the purified LAC immunoglobulins were not able to inhibit the maximal level of platelet-dependent prothrombinase activity. This difference might be due to a loss of specific antibodies during the purification procedure, since it had been found that polyclonal LAC IgG accounts for no more than the 0.2% of the whole IgG fractions (Pengo *et al*, 1987). Moreover protein(s) other than IgG might take part in the inhibitory process in the plasma of the patients (Exner *et al*, 1978).

In conclusion, our results indicate that LAC antibodies are able in plasma to react against procoagulant phospholipids from both platelets and extraneous sources. This reactivity depends more on the amount of phospholipids present in the system, than on their nature. Unlike Thiagarajan *et al* (1980), but in accordance with Dahlback *et al* (1983), we found no fundamental difference between the action of the inhibitor on platelet-mediated prothrombinase and on prothrombinase constituted with PS/PC vesicles. We do not exclude the possibility that patients may differ in this respect. In that case the patient studied by Thiagarajan *et al* seems to be an exception as in our series all nine patients reacted in the same way as the Dahlback patient.

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